FORM PTO-1390U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 5-93)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 9013.31

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/889645

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/00123	19 January 2000	19 January 1999

TITLE OF INVENTION

TREATING PROTEIN-CONTAINING LIQUIDS

APPLICANT(S) FOR DO/EO/US

McCallum et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).

6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [X] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.

8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.

14. [ ] A substitute specification.

15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information: International Preliminary Examination Report; International Search Report; PCT Demand; PCT Request.

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/889645	PCT/GB00/00123	9013.31

17. [X] The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO **$860.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) **$690.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$710.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$1,000.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT = $860.00	$ **860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	12 - 20 =	0	**X $18.00**	$	
Independent Claims	1 - 3 =	0	**X $80.00**	$	
Multiple dependent claim(s) (if applicable)			**+ $270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ **860.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ **860.00**	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ **860.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ **860.00**	
				Amount to be refunded	$
				charged	$

a. [X] A check in the amount of **$860.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-0220.

NOTE: **Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Commissioner for Patents, Washington, DC 20231.

Keowanna V C Best
Date of Signature: July 19, 2001

SIGNATURE

Robert J. Smith

40,820
REGISTRATION NUMBER

PTO FORM-1390 (REV 5-93)

Attorney's Docket No. 9013.31

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: McCallum, et al.
Serial No.: to be assigned
Filed: concurrently herewith
For: TREATING PROTEIN-CONTAINING LIQUIDS

Examiner: to be assigned
Group Art Unit: to be assigned

Date: July 19, 2001

PRELIMINARY AMENDMENT

DO/EO/US
Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to the examination of the above application and calculation of claim fees, please amend the above-identified application as indicated below. Attached hereto at page 4 is a marked-up version of the changes made to the specification and claims by the current amendment. The marked-up version of the changes is captioned "**Version With Markings To Show Changes Made**". The changes represent changes to the claims and specification from their amended form after the submission of amendments under Article 19 PCT.

In the Specification:

On page 1, line 1, please insert the following:

Cross-Reference to Related Applications

The present application is a U.S. national phase application of PCT International Application No. PCT/GB00/00123, having an international filing date of January 19, 2000 and claiming priority to Great Britain Application Nos. 9901139.7 filed January 19, 1999 and 9910476.2 filed May 7, 1999, the disclosures of which are incorporated herein by reference in their entirety. The above PCT International Application was published in the English language and has International Publication No. WO 00/43048.

In the claims:

4. (Amended) A Method according claim 1 wherein the solid porous particles are kieselguhr or perlite particles or mixtures thereof.

5. (Amended) A method according to claim 1 wherein the sold porous particles are diatomaceous earth particles.

7. (Amended) A method according to claim 1 wherein the pore size is in the range 0.6 to 6 microns.

8. (Amended) A method according to claim 1 wherein the pore size is in the range 0.6 to 1.5 microns.

9. (Amended) A method according to claim 1 wherein the depth filter has a thickness of 2 to 5 mm.

10. (Amended) A method according to Claim 1 wherein the natural product is a protein.

11. (Amended) A method according to claim 1 wherein the aqueous liquid comprises a blood plasma product.

12. (Amended) A method according to claim 11 wherein the blood plasma product is selected from the group consisting of albumin, an immunoglobulin, Factor IX, thrombin, fibronectin, fibringen, Factor VIII, Factor II, Factor VII, Factor IX, and Factor X.

REMARKS

Claims 1-12 are pending in the above application. Claims 4-5 have been amended to better conform to U.S. practice, and Claims 8-13 have been amended for the purposes of renumbering. Applicants respectfully request substantive examination on the merits.

Respectfully submitted,

Robert J. Smith
Attorney for Applicants
Registration No. 40,820

CERTIFICATE OF EXPRESS MAILING

"Express Mail" mailing label number EL733097607US
Date of Deposit: July 19, 2001
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Box Patent Application, Assistant Commissioner for Patents, Washington, DC 20231.

Keowanna V.C. Best
Date : July 19, 2001

Version With Markings To Show Changes Made

4. (Amended) A Method according **[to any preceding claim]** claim 1 wherein the solid porous particles are kieselguhr or perlite particles or mixtures thereof.

5. (Amended) A method according to **[any of claims 1 to 3]** claim 1 wherein the sold porous particles are diatomaceous earth particles.

[8.] 7. (Amended) A method according to claim 1 wherein the pore size is in the range 0.6 to 6 microns.

[9.] 8. (Amended) A method according to claim 1 wherein the pore size is in the range 0.6 to 1.5 microns.

[10.] 9. (Amended) A method according to claim 1 wherein the depth filter has a thinkness of 2 to 5 mm.

[11.] 10. (Amended) A method according to Claim 1 wherein the natural product is a protein.

[12.] 11. (Amended) A method according to claim 1 wherein the aqueous liquid comprises a blood plasma product.

[13.] 12. (Amended) A method according to **[claim 12]** claim 11 wherein the blood plasma product is selected from the group consiting of albumin, an immunoglobulin, Factor IX, thrombin, fibronectin, fibringen, Factor VIII, Factor II, Factor VII, Factor IX, **[or]** and Factor X.

TREATING PROTEIN-CONTAINING LIQUIDS

The present invention relates to a method for the removal of abnormal infective prion proteins associated with transmissable spongiform encephalopathies (TSEs) from an aqueous liquid containing natural products, especially biologically active proteins. The invention also relates to proteins (including foodstuffs and biologically active proteins)and medicinal compositions therefrom where the infective prion has been removed or inactivated.

There is concern about the potential transmission of TSEs such as Creutzfeldt-Jakob Disease (CJD) via whole blood or blood derived biopharmaceuticals. This concern has been heightened by a postulated link between bovine spongiform encephalopy (BSE) and a new variant form of CJD (vCJD)in humans. CJD is a progressive neurodegenerative disease caused by an unusual infectious agent that replicates in the lymphoreticular tissue and the central nervous system of its host. The nature of the agent is unresolved at present but two main hypotheses have been advanced. The first is the prion or infectious protein hypothesis; and the second is the virion hypothesis which encompasses a combination of host encoded protein with regulatory nucleic acid.

Medicinal compositions for intravenous infusion, intramuscular infusion and topical application have been prepared from human blood plasma for over four decades in a specialised but significant section of the pharmaceutical processing industry. A principal area of concern in the

safety of these products has been potential contamination with blood born viruses. However, the development of screening methods together with technology for the inactivation or removal of potentially contaminating viruses has greatly improved the safety of blood and preparations derived therefrom.

There is currently considerable concern about the possibility that biopharmaceutical products from human or animal sources may transmit TSEs. Although the precise nature of the infective agent in TSEs is at present unclear, TSEs such as Scrapie in sheep and CJD or vCJD in humans are associated with abnormal prion related proteins (PrPs). Suitable screening mentions have not yet been developed for abnormal PrPs, which are also extremely resistant to physical and chemical means of inactivation. For example, the EEC regulatory document (CPM Guidelines for Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathies by Medicinal Products: Biologicals, 20, pp155-158, 1992) recommends autoclaving at about 130°C for upto an hour, treatment with 1N sodium hydroxide for 1 hour or treatment with sodium hypochlorite for 1 hour. Such techniques are, however, quite unsuitable for the treatment of biologically active protein - containing materials since they result in total inactivation of the protein.

There is therefore a need to develop methods of removal or inactivation of abnormal infective prion proteins from animal, or human derived medicinal or food products which

are effective yet do not substantially degrade and/or remove the biological activity or food value of the product.

A major problem relates to the ill defined nature of the abnormal prion protein. The normal form of this protein is found in mammalian cells and is present in high levels in brain and lymphoreticular tissues. It is composed of highly membrane associated 33-35KDa phosphoinositol glycoprotein, which is completely sensitive to digestion with proteinase K. The infectious (abnormal) form of the protein has been shown to exist in an altered conformational form, contains a high level of β pleated-sheet, and is resistant to digestion with proteinase K. The change in conformation is thought to result in the protein becoming highly insoluble, forming aggregates which then deposit in the infected tissue as fibrils or amyloid plaques. The unknown properties of the abnormal prion proteins, and particularly the state of aggregation makes the prediction of suitable removal or inactivation techniques very difficult.

In the prior art, removal or inactivation by chromatographic techniques has been attempted. Hunter and Millson (J. Gen. Microbiol., 1964, vol. 37 pp251-258) showed examples of the chromatographic behaviour of scrapie-infected brain homogenate on DEAE cellulose adsorption and calcium phosphate columns. International patent application WO97/3454 relates to the removal of abnormal prion proteins from solutions of albumin or

reagent grade animal serum using typically expensive mixed ion exchange and hydrophobic solid phases.

Blum et al (BioPharm. 11(4) pp28-34, 1998) investigated the effectiveness of various steps (i.e. heating, precipitation, absorption with filter aid and ion-exchange chromatography) in the production of aprotinin and bovine serum albumin in removing added spikes of scrapie as a model source of TSE. However, it is unclear which of the above elements are essential or required in the removal of the scrapie agent.

Patent specification EP0798003 discloses filtration as a way of removing unwanted contaminants. A positively charged depth filter of 0.25 to 2.0 micron pore size also carrying a cation resin, was used for the removal of viruses from biologically active protein solutions. Morgenthaler (TSE issues, Cambridge Health Tech. Institute CHI, November, 1998, Lisbon, Portugal) has shown that filtration steps (including nanofiltration) can substantially remove added TSE spikes in the fractionation of human plasma.

It is an object of the present invention to further develop and characterise the removal of abnormal infective prion proteins from protein-containing liquids, particularly those derived from human plasma, without unacceptable effects on the nature or biological activity of the proteins.

It is a further object of the present invention to provide a depth filter which can be a single use filter and

may be disposed of having removed the abnormal prion proteins from the process stream.

The invention is based on the surprising discovery that filtration using a depth filter comprising particles and having a pore size less than six microns is surprisingly effective in removing abnormal infective prion proteins.

In particular, the invention provides a method for the removal of abnormal infective prion proteins associated with transmissable spongiform encephalopies (TSEs) from an aqueous liquid containing a natural product (especially a biologically active protein), which comprises passing the liquid through a depth filter formed of a matrix comprising solid particles of porous material and having a pore size providing a retention less than 6μm. Typically the filter may be a single use disposable filter.

By the term "removal" is meant the actual physical removal of the abnormal infective prion protein from the liquid containing the desired protein. For practical purposes, the recovery of the desired protein in its original biological state should be substantially maintained at least to a level in excess of 50%, preferably 80%, more preferably 90%.

Removal of the abnormal infective prion protein may be achieved to an extent of at least $10^{2.5}$, 10^3, preferably 10^4, more particularly 10^5.

The pore size of the filter matrix is preferably in the range 0.6 to 6 microns, particularly 0.6 to 1.5

microns. The pore size is defined in terms of the particle size of particles retained thereon. Typically particles of defined size such as micro-organisms are used for calibration purposes.

The invention also relates to the treated liquid.

Of particular importance to the fractionation of blood plasma products, is the discovery that filtration may be effectively carried out under non-denaturing conditions for the biologically active protein, and under conditions which do not reduce the solubility of the product protein. In addition filtration with or without filter aid can be used to remove suspended solids.

The method may be carried out at a pH in the range 4-10, preferably 5-9, and especially 6-8.

The application of heat is unnecessary and the process can be conducted at substantially room temperature or below, in particular in the range -5 to +20°C.

Preferably, the liquid and the filter are free of cationic or anionic charged material which may contribute to the reduction of biological activity of the biologically active protein, and in particular may cause activation of sensitive blood coagulation factors. The process is in particular applicable to the treatment of whole blood or liquids containing albumin, immunoglobulins, Factor IX, thrombin, fibronectin, fibrinogen, Factor VIII and Factor II, VII, IX and X and other proteins derived from plasma. It is also applicable to the treatment of plasma, Factor XI, Factor XIII, haemoglobin, alpha-2-macroglobulin,

haptoglobin, transferrin, apolipoprotein, mannan binding protein, protein C, protein S, caeruloplasmin, C-1-esterase inhibitor, inter-alpha-trysin inhibitor, Van Willebrand factor. Recombinant analogues of these may also be treated. In addition, the invention is applicable to the treatment of other natural products including foods, drinks, cosmetics etc. It is also applicable to other non-plasma animal-derived products, such as heparin and hormones.

The depth filter generally comprises a binder, such as cellulose, together with a solid porous particulate material such as Kieselguhr, perlite or diatomaceous earth.

The depth filter generally has a thickness in the region 1-10mm, particularly 2-5mm. The material used for the depth filter should have little or no effect on the desirable protein concerned.

Embodiments of the present invention will now be described by way of example only.

METHODOLOGY

1) Preparation of hamster scrapie spike

Hamster adapted scrapie (H_s) agent (strain 263K) was prepared by homogenisation of infected brain tissue in phosphate buffered saline. The titre of the agent produced in this way is normally of the order of 10^7-10^9 LD_{50} units ml^{-1} as assayed by the intracranial route in hamster. A stock of the hamster adapted scrapie strain agent (263K) is stored at or below -70C.

A microsomal fraction derived from crude brain homogenate was used for all spiking experiments.

The microsomal fraction was prepared according to the method of Millson et al (Millson GC, Hunter GD and Kimberlin RH (1971); "An experimental examination of the scrapie agent in the cell membrane mixtures. The association of scrapie activity with membrane fractions", J. Comp Path. 81, 255-265). Crude brain homogenate prepared from 263K infected brains by Dounce homogenisation was pelleted at 10,000g for 7min to remove nuclei, unbroken cells and mitochondria. The microsomes remaining in the supernatant were then pelleted by centrifugation at 100,000g for 90min, followed by resuspension in PBS.

2) Calculation of results

Clearance (C) and reduction (R) factors were calculated based on the end point dilution for samples after analysis by Western blotting. The end point dilution is calculated based on the first dilution at which no scrapie prion protein (PrP^{sc})can be detected. The reciprocal of this dilution is then taken as the titre of agent, and thus all titres are expressed in arbitrary units. Based on the titre determined by end point dilution, the total amount of PrP^{sc} in the sample is calculated based on the volume of the sample and taking into account any correction factors which need to be applied. Clearance factors are calculated relative to the theoretical input spike. Reduction factors are calculated

relative to the level of PrP^{sc} detected in the load sample.

Where no PrP^{sc} is detected at the highest concentration of sample tested, then the reciprocal of the dilution is taken as 1, and clearance and reduction factors are expressed with a ≥ sign proceeding the logarithmic value.

3) Western Blot Assay for Scrapie Infectivity

The titre of the stock of 263K used in this study, as well as the titre present in all samples generated during the study was determined by a Western blot procedure. This procedure relies upon the difference in susceptibility of the infectious (PrP^{sc}) and non-infectious (PrP^{c}) to proteinase K digestion. Samples were treated with protease K to digest away any PrP^{c}, and run on a SDS polyacrylamide gel followed by blotting onto nitrocellulose. Any PrP remaining after protease digestion, corresponding to PrP^{sc}, was then detected using a PrP specific antibody. The relative level of scrapie in the samples compared to the stock was determined by serial dilution to end point (the point where no signal was detected) of all samples.

Further information is given in A. Bailey, "Strategies for the Validation of Biopharmaceutical Processes for the Removal of TSE's", Cambridge Healthtech Institute, Nov.1998, Lisbon, Portugal.

Table 1 shows the efficiency of removal of spiked hamster scrapie prion proteins (PrP^{sc}) by various depth filters. Removal is expressed as clearance factor C (amount from innoculum/amount in filtrate) or as reduction

factor R (amount in feedstock/amount in filtrate). The Seitz KS80 filter of pore size 0.6 to 1.5 microns according to the present invention is highly effective in removing the prion proteins. Other filters presented for comparison purposes having either a larger pore size or including cationic species are less effective.

EXAMPLES

Example 1 (Treatment of Albumin according to the invention)

A model system was set up to replicate on an experimental scale the depth filtration of albumin in the conventional plasma fractionation process, employing different types of filter. The albumin-containing sample (fraction V) was spiked with hamster scrapie prion protein produced as described above and the concentration thereof was assessed by Western Blotting also as described herein.

The filter was a Seitz KS80 (trademark) pad cut to a 142mm diameter disk of effective filtration area 128cm^2. The filter was pretreated by passing ethanol and NaCl through for 35 minutes. The sample material was approximately 1 litre of resuspended fraction V at pH6.9 and 85.0g/l concentration taken from the conventional plasma fractionation process and kept at +4°C.

The conventional process involves the batch filtration of 853ml of sample. In this experiment, the same total volume of sample was passed through the filter, but only the final 100ml was spiked with microsomal hamster scrapie. 100ml of the sample starting material was spiked with 9.5ml

of the preparation of microsomal hamster (263k) scrapie and a sample of the spiked material was removed for analysis of the level of PrP^{sc}. The spiked material was passed through the filter at a flow rate of 6.4ml/min and the filtrate collected for analysis of the level of PrP^{sc}. All samples were stored at or below -70°C until analysis of the level of PrP^{sc} was carried out. Before the samples were analysed by Western Blotting, any scrapie in the sample was concentrated by ultracentrifugation.

Table 1 shows that the removal assessed by clearance factor C and reduction factor R exceeds four log units (the detection limit)with no abnormal prion protein being detected in the filtrate. The filter is therefore most effective in removing the added spike of hamster scrapie prion protein.

Example 2 (Albumin Treatment - Comparison)

The fraction V albumin-containing sample was filtered with a different filter in a similar manner to Example 1.

The filter used was a Cuno (trademark) Delipid Del 1 mini cartridge of effective filtration area 27cm^2. The filter was pretreated with ethanol and NaCl.

The sample material was fraction V at pH 6.9 and 85g/l concentration taken after conventional filtration through a Seitz KS80 filter and held at +4°C.

In this case, only the final 50ml were spiked with microsomal hamster scrapie. The spiked material was passed through the filter at a flow rate of 3.2ml/min and the

filtrate analysed for prion protein as before. The extent of clearance is shown in Table 1. Clearance and removal were only 2.8 and 2.3 logs respectively; abnormal prion protein being detected in the filtrate.

Example 3 (IGG - Comparison)

The procedure of Example 1 was repeated on IgG-containing supernatant I and III from conventional plasma fractionation.

The filter was a Millipore lifegard CP20 disk 47mm in diameter providing an effective filtration area of 12.5cm^2. The sample material was about 800ml of supernatant I and III (prefiltration) from the conventional plasma fractionation procedure and held at +4°C. It contained about 12% ethanol and had a pH 5.1.

680ml of sample was treated and the final 50ml was spiked with microsomal hamster scrapie.

The extent of removal of prion proteins is given in Table 1. The clearance factor C was 3.0 logs and the removal R was less than 1 log; abnormal prion protein being detected in the filtrate.

Example 4 (IGG - Invention)

The general procedure of Example 1 was repeated on IgG-containing fraction II suspension from conventional plasma fractionation.

The filter was a Seitz K200 (trademark) of 142mm diameter and effective filtration area 128cm^2. The sample

material was resuspended fraction II from conventional plasma fractionation held at +4°C.

600ml of sample was passed through the filter and the final 100ml was spiked with microsomal hamster scrapie. The extent of removal of the PrP^{sc} was determined as before and the results are given in Table 1. The clearance C and removal R both showed no abnormal prion protein in the filtrate to the limit of detection ($C \geq 3.4$ and $R \geq 2.8$).

Thus, it can be seen from the Examples that greater than 10^4 times removal of abnormal infective prion proteins can be achieved using a neutral filter having a pore size of less than 2 microns, and greater than $10^{2.5}$ times removal using a neutral filter of pore size 3.5 to 6.0 microns. These are the limits of detection. In other words no detectable abnormal prion protein was present in the filtrate.

Example 5 - (fibre filters-Comparison)

Four filters formed of fibres were tested for prion removal in a manner similar to Example 1.

Whole blood was spiked with 3.8 $\log_{10}$ of hamster scrapie (Hs 263K) and the clearance C and removal R determined.

The results given in Table 2 shows that these fibre filters gave < 1 log removal and were therefore ineffective for removing prion proteins.

TABLE 1

REMOVAL OF PrP^{sc} BY DEPTH FILTRATION

FILTER (PRODUCT)	COMPOSITION	RETENTION (μm)	C	R
Seitz, KS80 (Albumin)	Cellulose, Kieselguhr Perlite	0.6 - 1.5	≥ 4.1	≥ 4.9
Seitz, K200P (IgG)	Cellulose, Kieselguhr Perlite	3.5 - 6.0	≥ 3.4	≥ 2.8
Cuno, Delipid 1 (Albumin)	Cellulose, Kieselguhr Cation Resin	0.6	2.8	2.3
Millipore CP20 (IgG)	Borosilicate glass	2.0	3.0	<1 *

* Large drop in PrP^{sc} measured after addition of inoculum to process feedstock.

TABLE 2

FILTER (PRODUCT)	COMPOSITION	RETENTION (μm)	C	R
Baxter/Asahi Optiplus R7456(RS2000)	Negatively charged polyester fibres	n/a	< 1	< 1
Fresenius whole blood filter compoflex T2916	Melt blown woven-non-woven polyester fibre Non-ionic surface	n/a	< 1	< 1
Macopharma eucoflex LST-1	Nonwoven non ionic surface polyester/ polypropylene mixture 4 layers polyester/22 layers polypropylene/ 1 layer polyester	6-13	< 1	< 1
Pall leukotrap BPF-4	Non-woven polyester negatively charged	n/a	< 1	< 1

n/a - not available

CLAIMS

1. A method for the removal of abnormal infective prion proteins associated with transmissible spongiform encephalopies (TSEs) from an aqueous liquid containing a natural product, which comprises passing the liquid through a depth filter formed of a matrix comprising solid particles of porous material and having a pore size providing a retention less than 6μm.

2. A method according to claim 1 wherein the matrix comprises a binder.

3. A method according to claim 2 wherein the binder is cellulose.

4. A method according to any preceding claim wherein the solid porous particles are kieselguhr or perlite particles or mixtures thereof.

5. A method according to any of claims 1 to 3 wherein the solid porous particles are diatomaceous earth particles.

6. A method according to any preceding claim carried out in the absence of cationic or anionic charged material.

7. A method according to any preceding claim carried out at a pH in the range 4 to 10.

8. A method according to any preceding claim wherein the pore size is in the range 0.6 to 6 microns.

9. A method according to any preceding claim wherein the pore size is in the range 0.6 to 1.5 microns.

10. A method according to any preceding claim wherein the depth filter has a thickness of 2 to 5 mm.

11. A method according to any preceding claim wherein the natural product is a protein.

12. A method according to any preceding claim wherein the aqueous liquid comprises a blood plasma product.

13. A method according to claim 12 wherein the blood plasma product is albumin, an immunoglobulin, Factor IX, thrombin, fibronectin, fibrinogen, Factor VIII, Factor II, Factor VII, Factor IX, or Factor X.

14. A liquid subjected to prion removal according to the method of any preceding claim.

Attorney's Docket No. 9013-31 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: (*check one applicable item below*)

- ☐ original
- ☐ design
- ☐ supplemental

NOTE: *If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.*

- ☒ national stage of PCT

NOTE: *If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.*

- ☐ divisional
- ☐ continuation
- ☐ continuation-in-part (C-I-P)

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

TREATING PROTEIN-CONTAINING LIQUIDS

SPECIFICATION IDENTIFICATION

the specification of which: *(complete (a), (b) or (c))*

(a) ☐ is attached hereto.

(b) ☐ was filed on _____ as ☐ Serial No. 0 /_____
or ☐ Express Mail No., *as Serial No. not yet known* _____
and was amended on _____ (*if applicable*).

NOTE: *Amendments filed after the original papers are deposited with the PTO which contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67.*

(c) ☒ was described and claimed in PCT International Application No. PCT/GB00/00123 filed on 19 January 2000 and as amended under PCT Article 19 on _____ (*if any*).

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information

☐ which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56

(also check the following items, if desired)

☐ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☐ In compliance with this duty there is attached an information disclosure statement in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☐ no such applications have been filed.

(e) ☒ such applications have been filed as follows.

NOTE: *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

A. PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
GB	9901139.7	19 01 1999	☒ YES ☐ NO
GB	9910476.2	7 05 1999	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

NOTE: *If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.*

POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (*List name and registration number*)

☒ Attached as part of this declaration and power of attorney is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO

20792
PATENT TRADEMARK OFFICE

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

Robert J. Smith
919-854-1411

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: *Carefully indicate the family (or last) name as it should appear on the filing receipt and all other documents.*

1-00 **Full name of sole or first inventor**

ANNE *(GIVEN NAME)* GILLIAN *(MIDDLE INITIAL OR NAME)* WELCH *FAMILY (OR LAST NAME)*

Inventor's signature Anne S Welch

Date 23.07.01 **Country of Citizenship** United Kingdom GBX

Residence United Kingdom

Post Office Address 31 The Firs, Dalgety Bay, Fife KY11 9UH, United Kingdom

2-00 **Full name of second joint inventor, if any**

PETER *(GIVEN NAME)* REYNOLDS *(MIDDLE INITIAL OR NAME)* FOSTER *FAMILY (OR LAST NAME)*

Inventor's signature P R Foster

Date 17 Jul 2001 **Country of Citizenship** United Kingdom GBX

Residence United Kingdom

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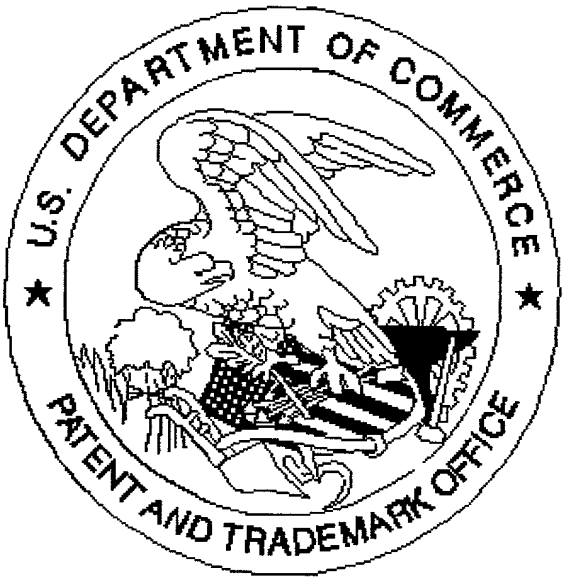
United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☒ Page(s) 5 of declaration were not present for scanning.
(Document title)

☐ Page(s)_____ of_____ were not present for scanning.
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